EXHIBIT A80

Hysterosalpingo-Radionuclide Scintigraphy (HERS)

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A radionuclide procedure, hysterosalpingo-radionuclide scintigraphy (HERS), was designed to evaluate the migration of a particulate radioactive tracer from the vagina to the peritoneal cavity and ovaries as well as to image and functionally outline the patency of the pathways between these two extremes of the female reproductive system. Technetium-99m human albumin microspheres (^{99m}Tc-HAM) were deposited in the posterior fornices of patients who were divided into two specific groups. Group I consisted of patients who were to undergo different elective gynecologic operations, in which besides obtaining sequential images, radioactivity levels were measured in the removed organs and tissues. Group II consisted of patients referred by the Infertility Clinic for evaluation of their reproductive system pathways patency. In this latter group, HERS was compared with contrast hysterosalpingography (HSG) and peritoneoscopy (PCP). The results obtained from measurements of radioactivity levels on the removed surgical specimens and comparison with other conventional gynecologic diagnostic procedures provide accurate evidence of the migration of ^{99m}Tc-HAM from the vagina, through the uterus and tubes, to the peritoneal cavity and ovaries, and show that HERS is a simple noninvasive method for functionally imaging and assessing the patency of the female reproductive system pathways.

IN THE adult female, the peritoneal cavity communicates with the outside via the fallopian tubes, the uterus, and the vagina and there is evidence for the migration of different substances in either direction (Fig. 1). For example, malignant cells from ovarian carcinoma can be demonstrated in the posterior fornix of the vagina.[1] After menstruation, the gonococcus can penetrate the cervix and gain access through the uterus and tubes to the peritoneal cavity and ovaries.[2] Retrograde menstruation is also a well known phenomenon. For pregnancy to occur, spermatozoa have to move up the uterus as the ova moves down the tube. After insufflation, air and gases pass easily from the vagina into the peritoneal cavity up to the diaphragm. Radioopaque contrast media are introduced with great ease through the uterus and tubes into the peritoneal cavity, and tubal patency is easily demonstrated during peritoneoscopy by injection of a dye through the cervix and into the tubes.

If transit can take place so easily, it is probable that the same happens with chemical substances used for hygienic, cosmetic, or medicinal purposes, many of which may have potential carcinogenic or irritating properties (Table 1). Such migration could well explain the etiologic role of chemical substances in certain gynecologic diseases, and specially in carcinoma of the ovary.[3-5] A role for environmental factors and socioeconomical conditions in the origin of ovarian carcinoma has been inferred from its higher incidence in industrialized countries[6] (Table 2). The incidence of carcinoma of the ovaries in South African whites is substantially higher than in South African blacks.[5]

The products of industry upon which most attention has been focused are asbestos and talc. Whereas the carcinogenic properties of asbestos are undisputed,[7] there is still controversy over talc.[8] Although conclusive data are lacking, various facts indicate that talc could be a possible carcinogen, cocarcinogen, or promoter of malignant transformation, and should not be used as a dusting powder.[9] This is based on the fact that talc, a hydrous magnesium silicate [$Mg_6S_{18}O_{20}(OH)_4$] is chemically similar to asbestos, which is a calcium magnesium silicate [$Ca_2MG_5S_{18}O_{22}(OH)_2$]; besides, talc frequently contains microscopic fibers of asbestos as a contaminant.[10]

Access of talc to the peritoneal cavity is most likely through the vagina. Studies of the transport of particles in the human female reproductive tract have shown that nonmotile inert carbon particles deposited in the vagina can be recovered 30–35 min later in the fallopian tubes.[11]

Electron micrographic slides of removed human ovaries have shown asbestos particles resting on them, and there is evidence that these

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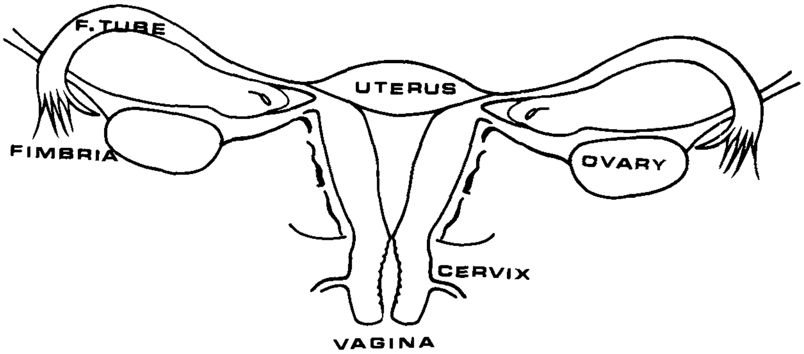


Fig. 1. Schematic representation of the female reproductive system pathways seen from in front.

particles originated from talc used to dust condoms.[12] In this circumstance, talc particles were probably thrusted by the penile pumping action during intercourse. Furthermore, Henderson et al. found talc particles deeply embedded in 75% of ovarian tumors studied.[13,14]

The potential harmful effects of talc on a highly differentiated tissue such as the ovary, with its interrelated cell types and cyclical changes of secretory activity, should certainly not be ignored.[15]

To demonstrate the upward migration of nonmotile, inert chemical substances we made use of radionuclide imaging and counting techniques.[16] During the course of the study, we came to recognize that the value of the images obtained outlining the female reproductive system pathways functionally reflected the dynamic state of this system and could be used as an additional and/or alternative diagnostic modality in clinical gynecologic practice in evaluating tubal patency. Diagnostic procedures where gases, fluids, dyes, and contrast medium are introduced through manual interventions under positive pressure from the uterine cervix into the peritoneum, are anatomically accurate and safe in the hands of those performing them regularly, but do not physiologically portray

Table 1. Possible Chemical Carcinogens Used in the Vagina for Cosmetic, Hygienic, and Medicinal Purposes*

1	Arsenicals
2	Hydroxiquinolines
3	Nitrofurantion
4	Ichthammol
5	Sulphonamides
6	Metronidazole
7	Nitrosamine†
8	Spermicides
9	Asbestos‡
10	Talc
11	Gentian violet

*From Venter.[5]
†Possible formation by chemical reduction.
‡As a contaminant.

Table 2. Incidence of Carcinoma of the Ovaries in Different Countries (per 100,000)*

Sweden	21.0
Norway	16.5
USA (whites)	15.6
England	14.7
Israel	11.0
USA (blacks)	8.8
USA (hispanics)	5.9
Africa	4.6
India	3.2
Japan	3.1

*From Kolstad and Beecham.[6]

Table 3. Surgical Indication and Operative Procedure (Group I)—24 Patients

No. Patients	Surgical Indication	Operative Procedure
4	Sterilization	Fimbriectomy
7	Ca. breast stage III	Bilateral salpingo-oophorectomy
1	Ca. breast stage III	Hysterectomy and bilateral salpingo-oophorectomy
2	Postmenopausal bleeding	Dilatation and curettage
2	Postmenopausal bleeding	Hysterectomy and bilateral salpingo-oophorectomy
3	Menorrhagia	Dilatation and curettage
4	Menorrhagia	Hysterectomy and bilateral salpingo-oophorectomy
1	Pelvic infection	Hysterectomy and bilateral salpingo-oophorectomy

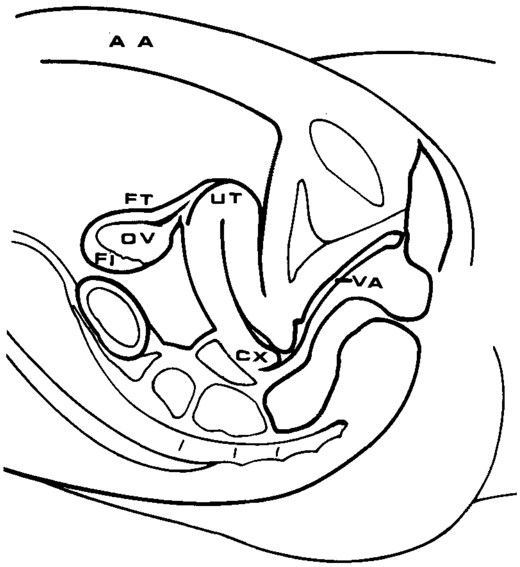


Fig. 2. **Median sagittal section of female genitalia to show relationships in the position in which the study was carried out. AA, anterior abdominal wall; VA, vagina; CX, cervix; UT, uterus; FT, fallopian tube; FI, fimbria; OV, ovary.**

fallopian tube patency. They are invasive procedures, uncomfortable for the patient, restricted under certain conditions, and not free of risks of hypersensitivity reactions inherent in any contrast medium.

MATERIALS AND METHODS

Patients in this study were divided into two different groups. Group I consisted of 24 adult women, both blacks and whites, admitted to hospital for elective gynecologic operations (Table 3). Group II consisted of 29 young white adult women referred by the Infertility Clinic for evaluation of their tubal patency. The radionuclide procedure was explained and the necessary consent was obtained.

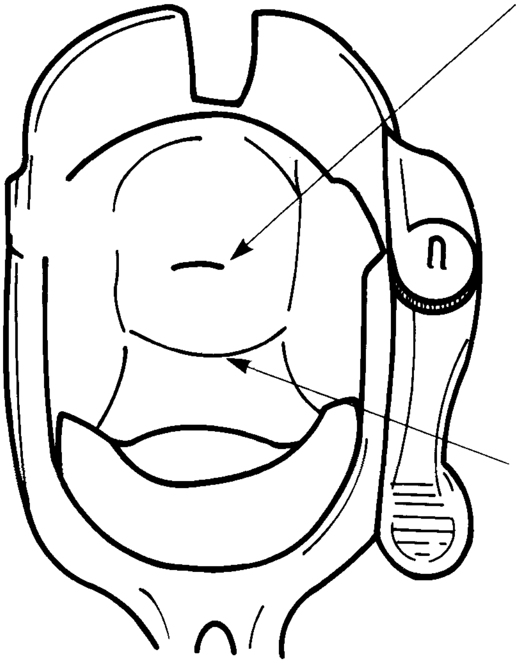


Fig. 4. **Exposed cervix seen from in front with arrows showing external cervical os and posterior fornix where ^{99m}Tc-HAM is usually deposited during HERS.**

Procedure

The patient was placed in the supine gynecologic examination position with the buttocks slightly elevated or in the Tredelenburg position. (Fig. 2). The cervix and posterior fornix were exposed with a Cusco vaginal speculum (Fig. 3) and 10 mCi (for patients of group I) and 2–3 mCi (for

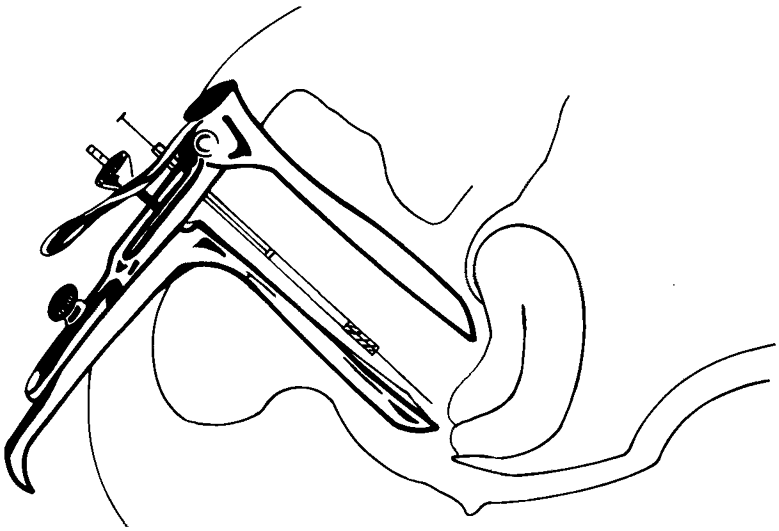


Fig. 3. **Cervix exposed with a Cusco vaginal speculum and syringe in place for deposition of ^{99m}Tc-HAM for HERS.**

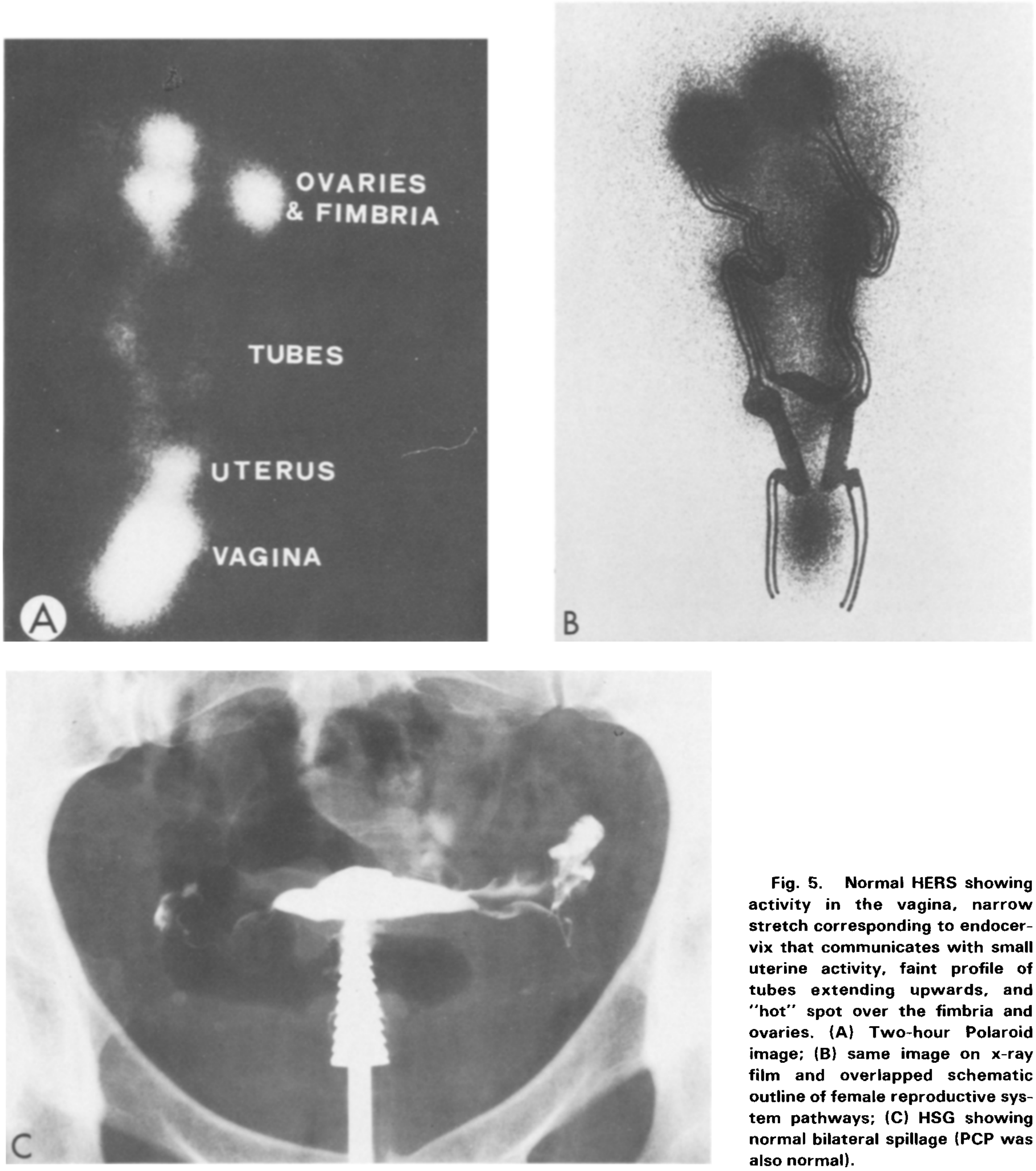
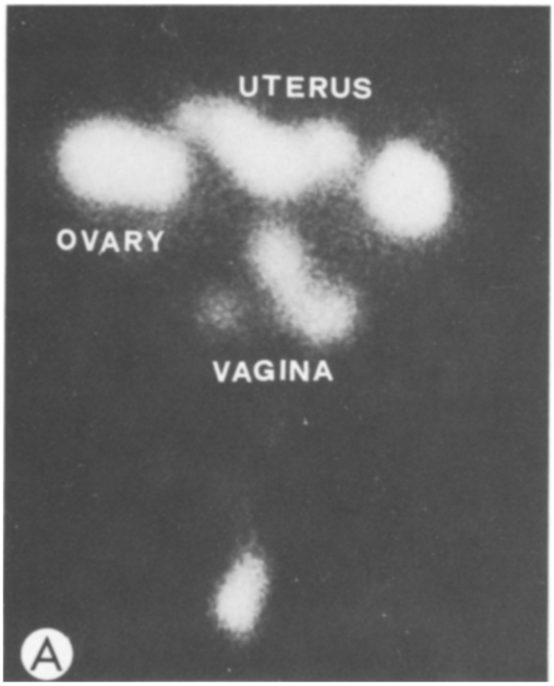
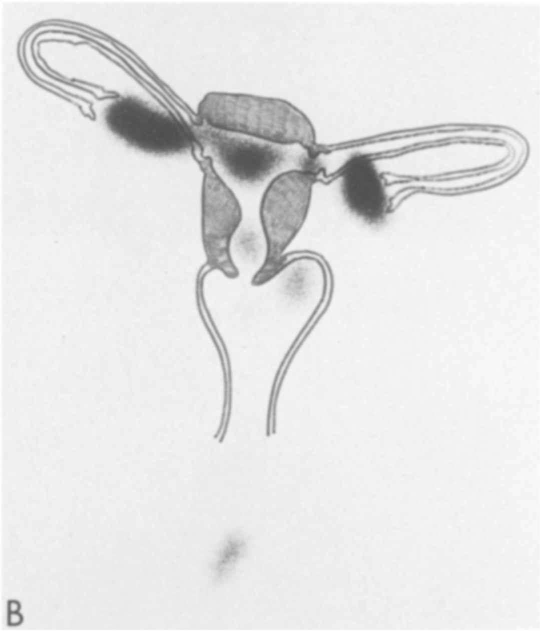


Fig. 5. Normal HERS showing activity in the vagina, narrow stretch corresponding to endocervix that communicates with small uterine activity, faint profile of tubes extending upwards, and "hot" spot over the fimbria and ovaries. (A) Two-hour Polaroid image; (B) same image on x-ray film and overlapped schematic outline of female reproductive system pathways; (C) HSG showing normal bilateral spillage (PCP was also normal).

patients of group II) of ^{99m}Tc-HAM in a volume of less than 1 ml were deposited in the posterior fornix, or close to the cervical external os (Fig. 4). The plastic cover of the needle (37 mm) was kept in place so as not to accidentally hurt the exposed tissue. The radionuclide was quickly discharged and the vaginal speculum carefully withdrawn while trying not to let the radioactive fluid leak out from the vagina. The vulva was then covered with a sanitary towel and the legs pressed or crossed together. The patient was kept in this position for the next 3 hr.

In patients from group I, about 24 hr after deposition of the radioactive tracer in the vagina, counts were performed on removed surgical specimens using a 12.7 cm well-scintillation detector. Where the uterus and adnexae were removed together, they were first counted as a whole and later separately. In the five patients that had D & Cs, only the endometrial scrapping was counted. In the case of fallopian tubes, each one was counted separately and the fimbria and ovaries separately from the isthmus. In two cases, a piece of the anterior peritoneum, fluid from the pouch of Douglas, peripheral blood, and lymphatic glands were also counted to determine the possibility of reabsorption of the radionuclide





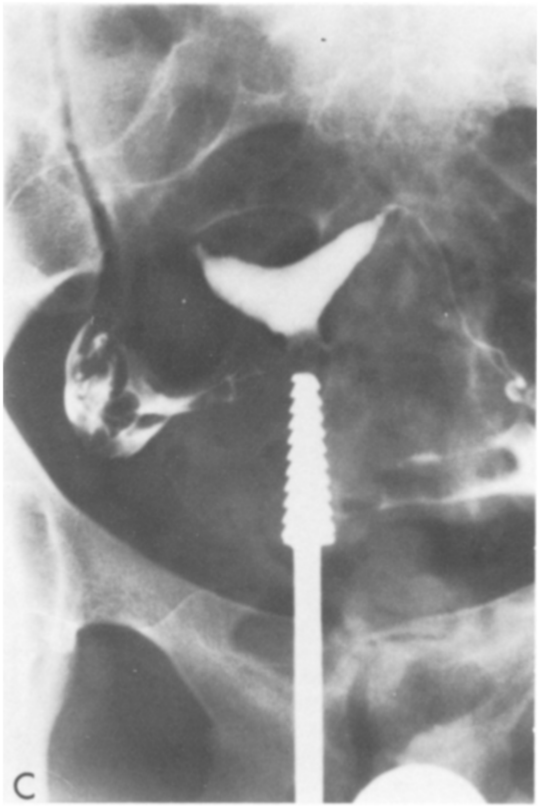


Fig. 6. Normal HERS; bicornate uterus with tubes extending laterally. (A) Three-hour Polaroid image; (B) same image with overlapped schematic outline of female reproductive system; (C) normal free spillage on HSG (PCP reported patent tubes).

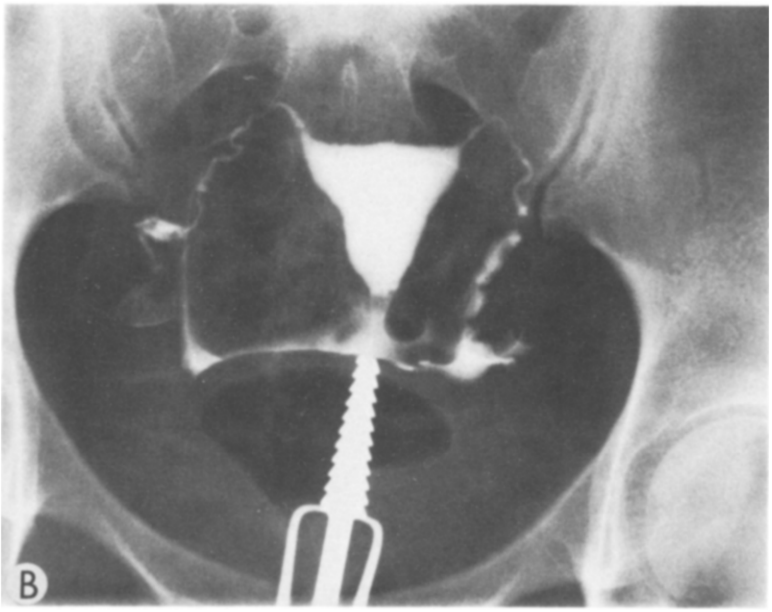


Fig. 7. (A) Normal HERS 1, 2, and 24 hr after deposition of ^{99m}Tc-HAM in posterior fornix of the vagina. (B) Patent tubes reported on HSG and PCP.

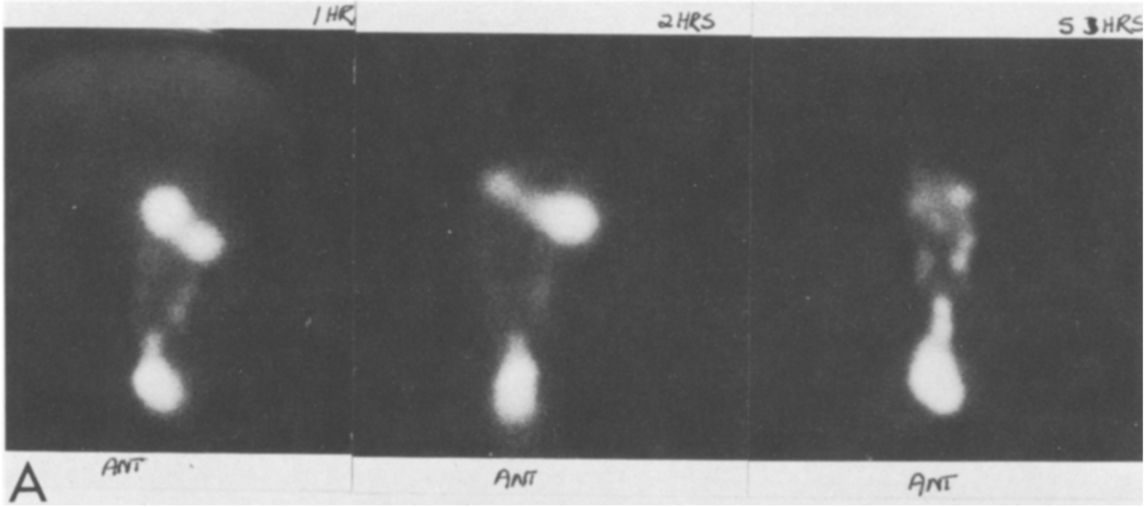
into the blood stream or lymphatic drainage from the vaginal mucosa.

If radioactivity levels measured on the removed surgical specimens were substantially higher than background levels, this constituted positive evidence of migration of the ^{99m}Tc-HAM from the vagina to the uterus or the tubes and ovaries. However, if radioactivity levels measured were comparable to background levels, it was taken as evidence that no migration of ^{99m}Tc-HAM had taken place and the cause for this possible obstruction was investigated.

Images were obtained 1, 2, 3, and 24 hr after deposition of the radioactive tracer on a large field of view gamma camera with a low-energy parallel all-purpose collimator, to a total of 400–500 K counts. The usual was an anterior view over the lower pelvic region, and in selected cases, images were also obtained shielding the high activity in the vagina in order to enhance the image of the uterus and tubes. Scintiphotos were recorded on Polaroid and x-ray film.

The normal pattern of the images obtained with this procedure would be a central elongated area of high activity over the vagina. Directly on top of this area would be a narrow stretch of activity corresponding to the endocervix, which would communicate the vagina and intrauterine activity. The uterus appeared as a smaller area of varying size, position, and shape (in most cases it was triangular). The tubes would be seen extending laterally or upward in a diverging angle with a distal "hot" spot of high intensity corresponding to the fimbria and ovaries (Fig. 5). In some cases, activity in the region of the tubal isthmus could not be visualized, although there was high activity in their distal segment (Figs. 6 and 7). In most cases, activity progressed within the first hour simultaneously through both tubes, while in others, activity moved faster in one tube than in the other, showing increased activity on one side. (Fig. 8). Scans were interpreted as abnormal if there was no activity in one or both tubes and specially if the distal focal area of high activity in the fimbria did not show up (Figs. 9, 10, and 11). Anatomic variants were also detectable (Fig. 12).

All patients of group II also had contrast hysterosalpingography (HSG) and peritoneoscopy (PCP) done after HERS. Spillage of the contrast media into the peritoneal cavity during HSG or appearance of the dye in the fimbria during PCP was an evident sign of tubal patency. The pressure exerted to introduce these substances from the



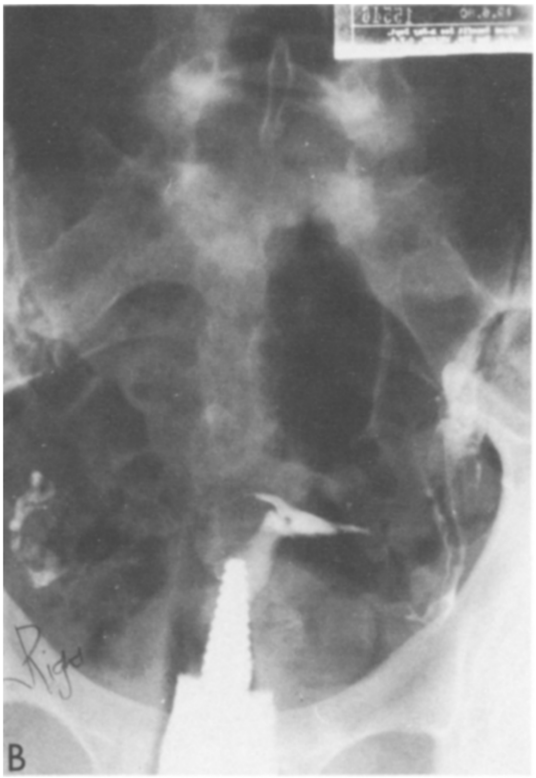


Fig. 8. (A) Normal HERS. Asymmetrical pattern of flow seen in 1, 2, and 5 hr images. (B) Both tubes reported as patent on HSG and PCP.

uterine cervix to the peritoneal cavity was also taken into consideration. Results of the three diagnostic procedures were later compared and clinically evaluated. (See Results below.)

Radiation exposure to patients of group I was low or in most cases negligible, since the target organs had been surgically removed. However, this was not the case for patients of group II who were sexually active and in potentially childbearing age.

We were concerned because the radioactivity reaching the fimbria and ovaries, which in this case were the target organs, decayed there physically, as there is no know mechanism for the biologic removal of the ^{99m}Tc-HAM once they reach the critically radiosensitive gonads. For this reason, we reduced the dose of the deposited ^{99m}Tc-HAM in the vagina to 2–3 mCi during the course of the study of patients from group II without sacrificing clinically informative value to the procedure.

Fortunately, most of the deposited radioactivity appears to be in the vagina and only a fraction of it migrates to the

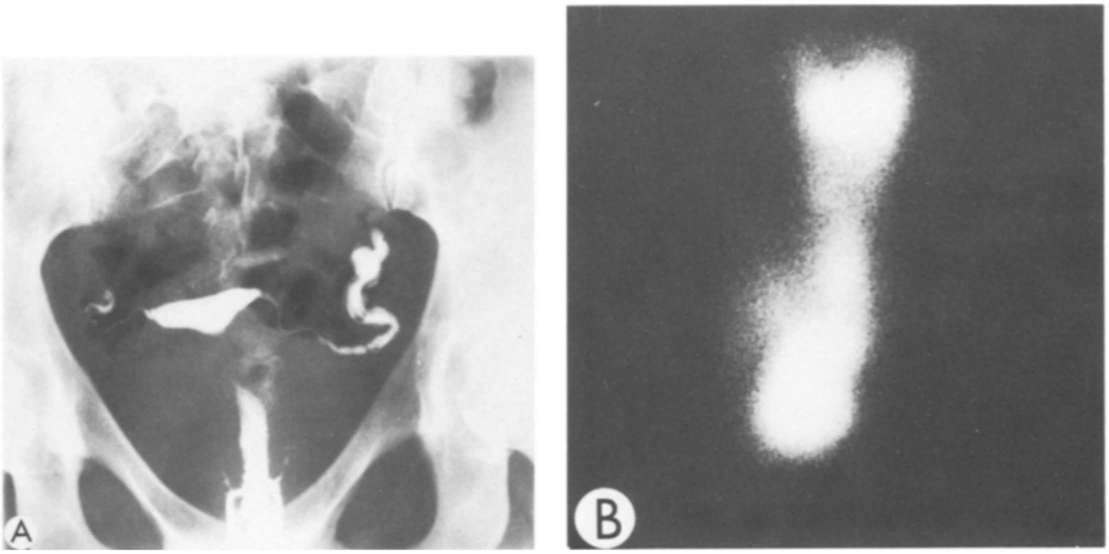


Fig. 9. **Patient with left side hydrosalpinx. (A) HSG shows a dilated and contorted left tube with a short and thin right tube. There was spillage in the left side with obstruction in the right side tube. (B) A 2-hr image of HERS shows the same pattern with no migration of ^{99m}Tc-HAM in the right tube.**

uterus and tubes (Fig. 13). Furthermore, in most cases this migration occurs within the first 3 hr and no further imaging is needed at 24 hr, which makes it possible to still obtain good quality images while reducing the radiation dose to the patient to safer levels comparable to those of x-ray diagnostic procedures.[17]

RESULTS

Because the radioactive material leaked out from the vagina in 3 patients, these patients were excluded from the final analysis of the 24 patients of group I (Table 4). In 16 of the

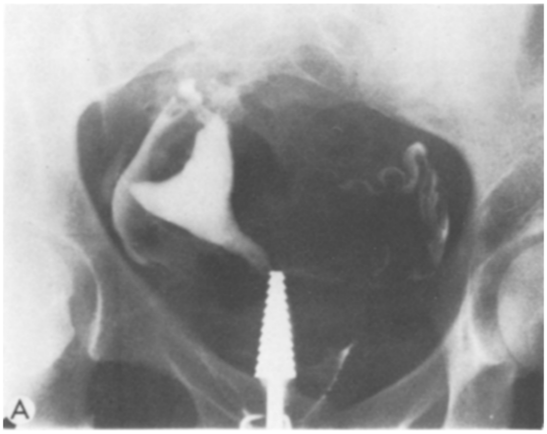
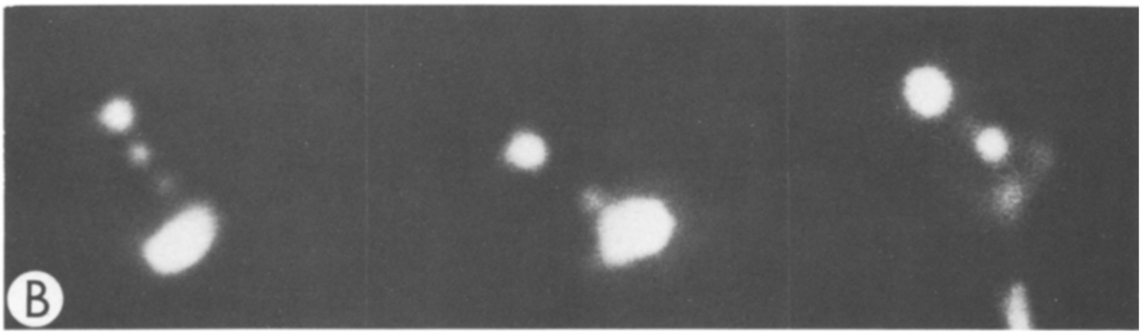


Fig. 10. **HERS and HSG (A) show uterus displaced to the right with long contorted left tube and obstructed right tube. (B) HERS on the 2, 3, and 24 hr images show focal "droplets" of higher activity at site of prominent kinks of left tube.**



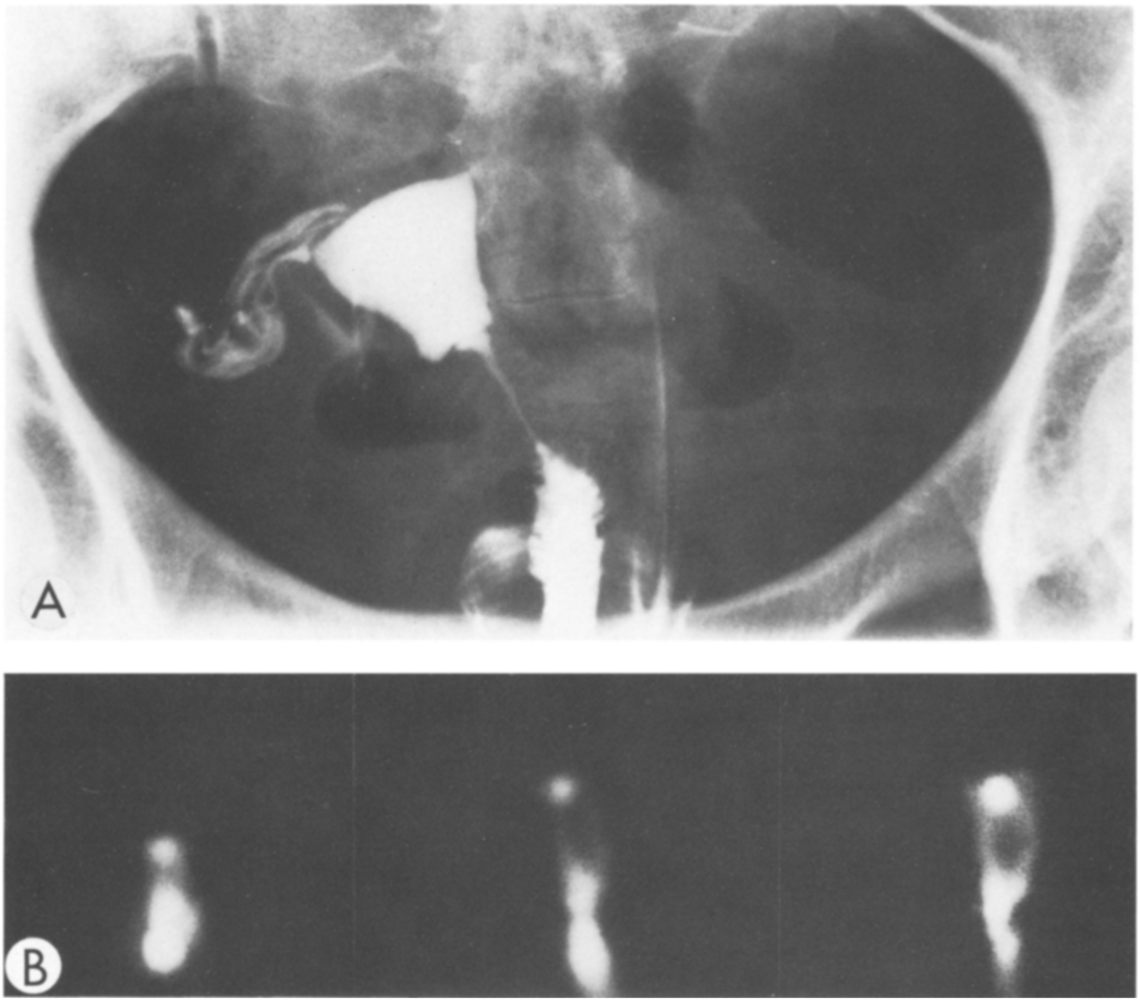


Fig. 11. (A) HSG shows right tube to be patent while the left tube is only seen in its proximal segment. (B) HERS shows the same pattern at 1 and 2 hr. Later, at 24 hr, activity can be seen migrating through left tube but not reaching the fimbria.

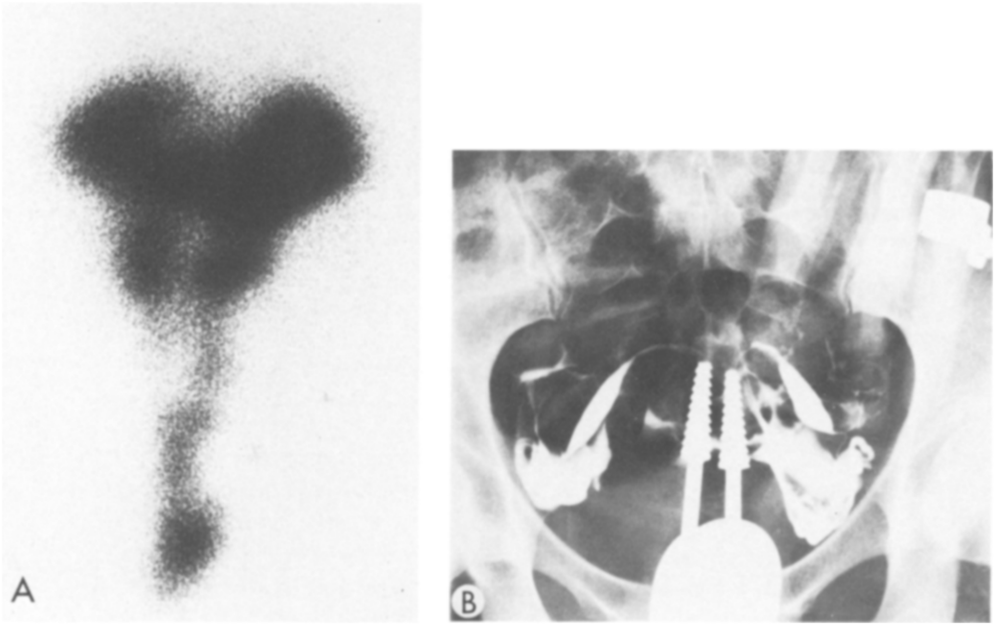


Fig. 12. Patient with didelphos as outlined on 1-hr image of HERS (A) and HSG (B).

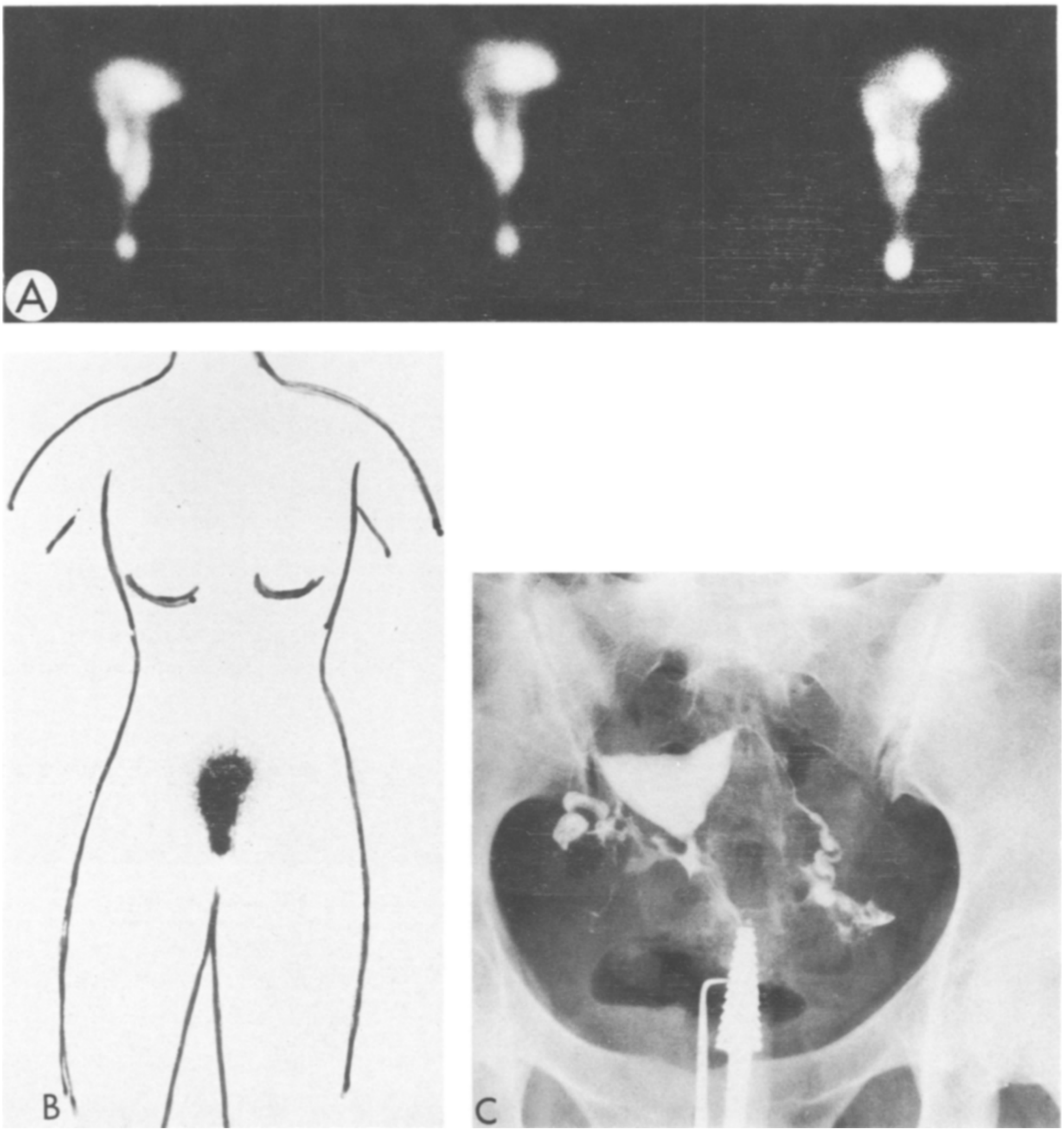


Fig. 13. (A) HERS shows a normal pattern of migration on 2, 3, and 24-hr images. (B) Twenty-four-hour whole body scan shows activity exclusively in the area of interest. (C) Bilateral tubal patency reported on HSG and PCP.

Table 4. Summary of Results (Group I)

Positive migration	16
Negative migration	
No passage to uterus	2
No passage to adnexae	3
Technically defective	3
Total patients examined	24

Table 5. HERS Versus HSG and PCP (Group II)

Agreement between HERS, HSG, and PCP	21
Disagreement between HERS/HSG and PCP	
HERS (−); HSG and PCP (+)*	5
HERS (+); HSG and PCP (−)	1
Technically defective	2
Total patients examined	29

*Tubes patent (+); tubes not patent (−).

remaining 21 patients there was positive evidence of migration of the ^{99m}Tc-HAM from the vagina to the uterus or the tubes and ovaries. The results were negative in 5 cases; in 2 of them the radioactive ^{99m}Tc did not pass from the vagina to the uterus, and in the other 3 there was no migration to the adnexae or fimbria.

In 14 of 21 cases, it was possible to measure high radioactivity levels in the adnexae separately from the uterus. Nine of these showed marked radioactivity in the tubes and ovaries (most of it localized in the fimbria). In 5 cases, radioactivity levels in the tubes were not much higher than the background, and in these patients severe tubal occlusion due to previous infection was confirmed by pathologic study of the surgically removed specimens. In the two patients where pieces of the anterior peritoneum, peripheral blood, and lymphatic glands were counted, the radioactivity levels of the samples were as low as that of the background. This showed that the ^{99m}Tc-HAM had not reached the adnexae through the blood supply owing to local reabsorption or lymphatic drainage from the vaginal mucosa where they had been deposited.

When HERS was compared with the results of HSG and PCP in group II (Table 5), we found that in 21 patients there was complete accordance between the 3 diagnostic modalities, be it that the tubes were patent or occluded. In one case, HSG and PCP showed that the tubes were patent, while initially HERS showed no migration in one tube during the first 3 hr of observation, but this changed later at 24 hr, when radioactivity appeared in the distal end of that tube (Fig. 14). In 6 cases there was no agreement between HERS and HSG and PCP. In 5 of them, both HSG and PCP showed that the tubes were patent when the contrast media and the dye were introduced under extreme pressure (Figs.

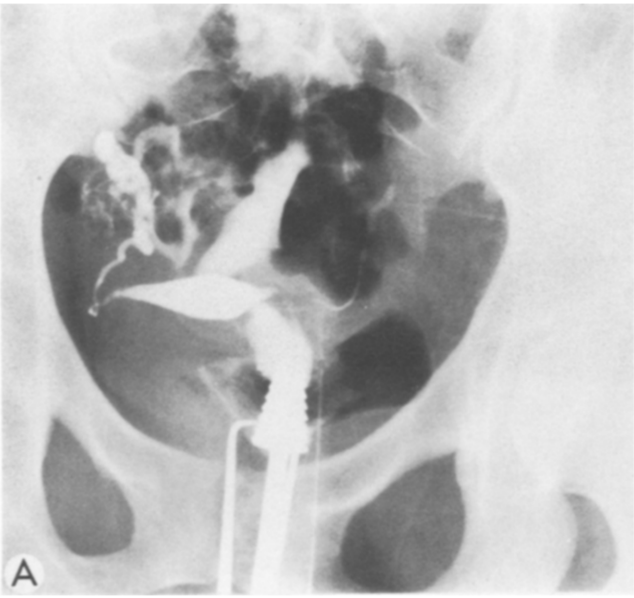
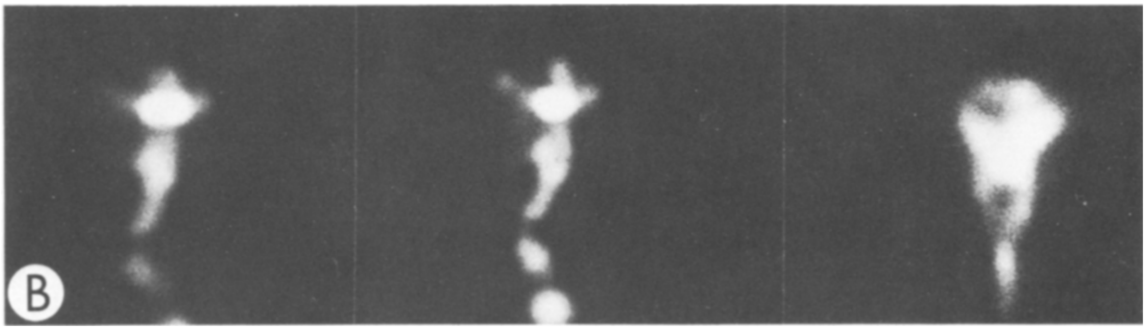


Fig. 14. (A) HSG shows bilateral tubal patency. (B) Two hour and 3 hr images on HERS show migration on left tube only, which appears to be long and contorted. At 24 hr, radioactivity appears to have migrated through right tube as well.



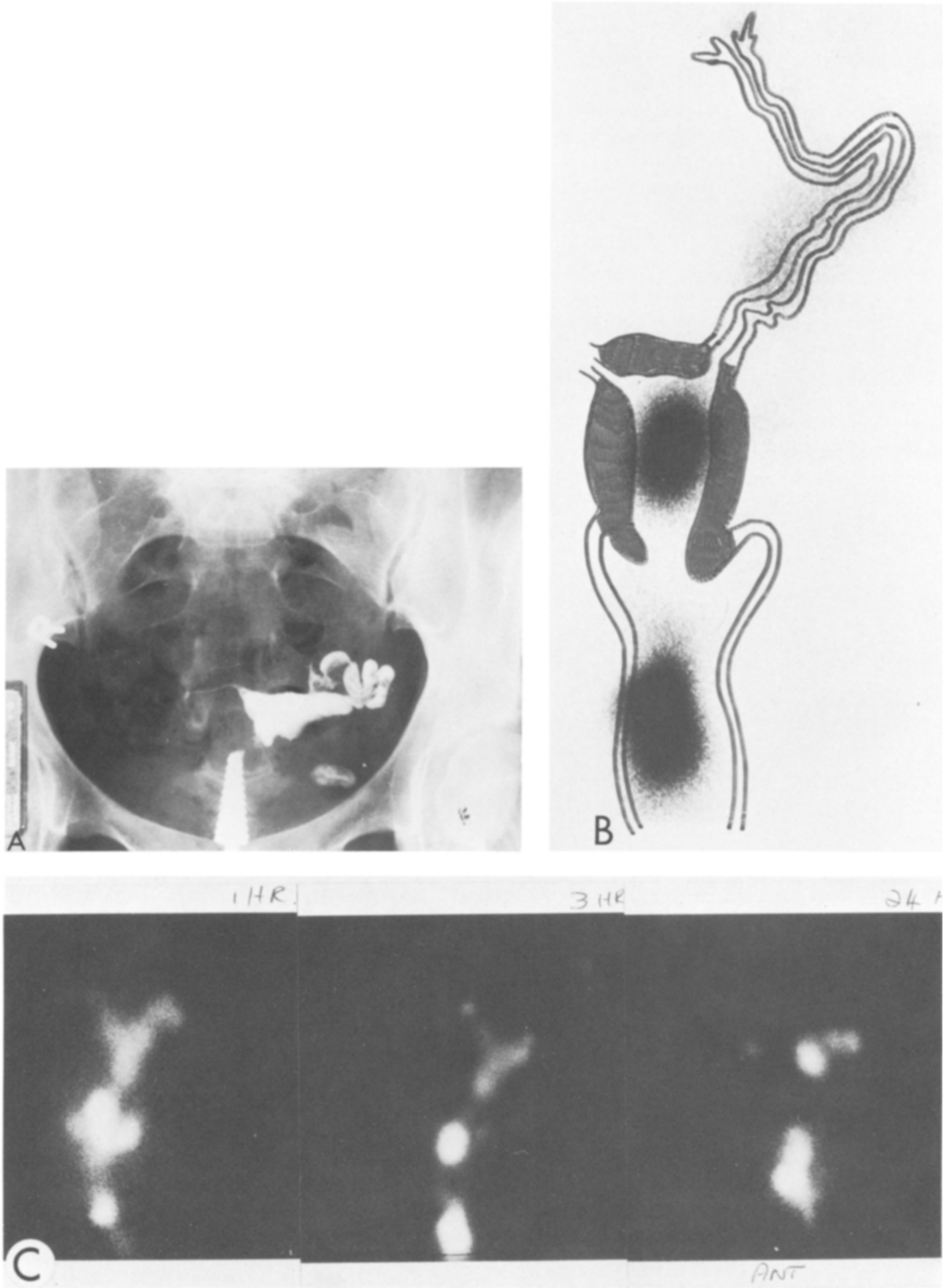


Fig. 15. (A) During HSG and PCP both tubes were reported to be patent, but only after introducing contrast media and dye, respectively, under extreme pressure. (B and C) HERS shows that up to 24 hr, there is no migration through the right tube, while the left tube appears long and kinked.

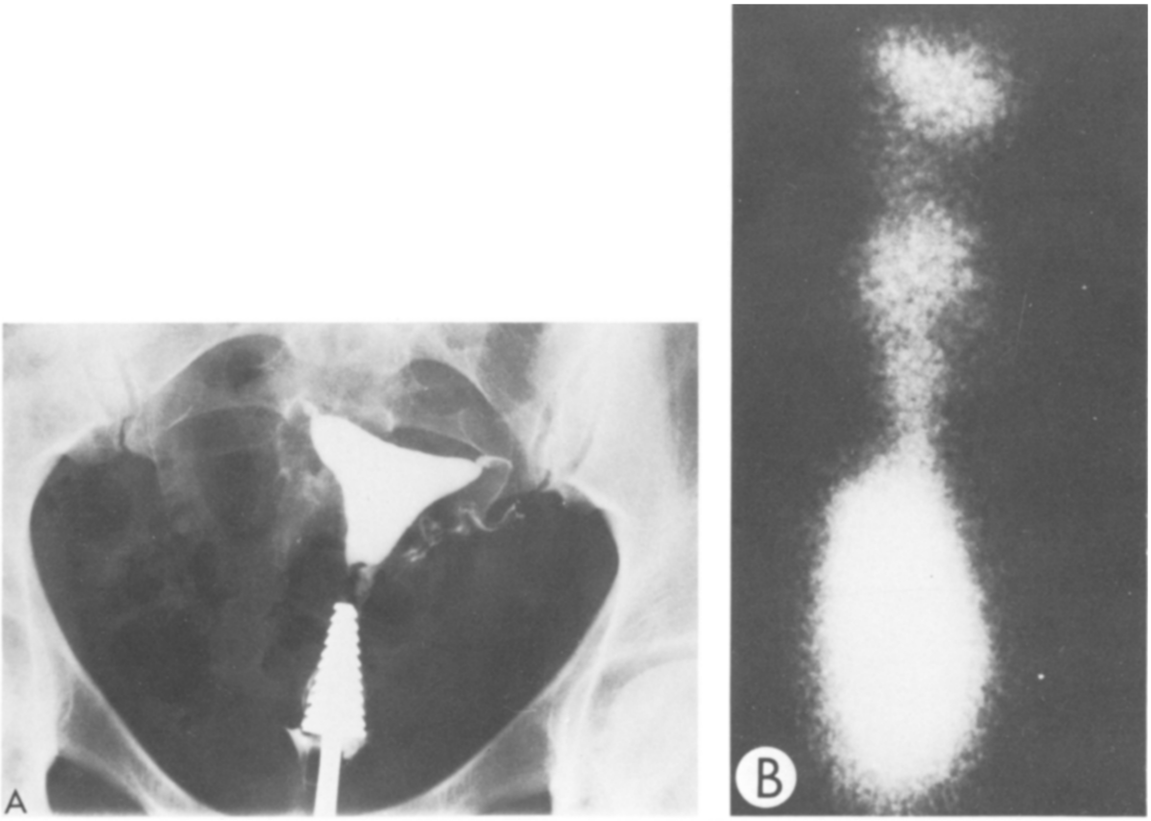


Fig. 16. (A) During HSG and PCP, both tubes were reported to be patent, but only after introducing contrast media and dye, respectively, under extreme pressure. (B) On the 24-hr image, HERS shows no migration of ^{99m}Tc-HAM through the right tube.

15 and 16). In these 5 cases, HERS showed no evidence of migration in one or the other tube, reflecting in this way the physiologic state of the fallopian tubes. In only one case did HERS show patency in one tube, while HSG and PCP did not, this was in the case of a woman with a septum in her vagina and a double uterus where manipulations for HSG and PCP were difficult. Finally, in 2 cases the results were equivocal because at least 2 of the 3 diagnostic procedures were technically deficient and no clinical information of diagnostic value could be obtained.

DISCUSSION

The results obtained from HERS in patients from group I clearly demonstrate the upward migration of a particulate radioactive tracer such as ^{99m}Tc-HAM from the vagina through the uterus and tubes into the peritoneal cavity and ovaries. This evidence correlates with findings on the surgically removed specimens, proving the accuracy of this radionuclide procedure. The real importance of this finding is that it supports previous evidence for the migration of inert, nonmotile chemical substances from the vagina to the peritoneum and ovaries,[11–14] and could help explain the role that some of these apparently innocent and frequently used substances play in the etiology of certain gynecologic diseases.[3,4,8,9]

The mechanism by which this migration takes place is not clearly defined, but it is assumed that it is a combination of muscular peristaltic movements, changes in peritoneal pressure, and ciliary motion (in the tubes) that drives particles from the vagina to the peritoneum and ovaries. The abundance of blood vessels interspersed with muscle bundles and active mucosal secretion form in the fimbria a kind of erectile tissue where most of the tubal activity tends to gravitate. There must also be a cyclical hormonal component regulating this process, and we

presume that migration is facilitated during the period of ovulation.

As far as the radionuclide imaging process is concerned, it was encouraging to find a close correlation of this procedure when compared with HSG and PCP. But most important of all is the fact that HERS functionally reflects the dynamic state of the female reproductive system pathways by showing particulate migration, which is not the case of the other anatomically dependant diagnostic modalities used to evaluate tubal patency. In this small series we found that in five cases, HSG and PCP were reported showing anatomical tubal patency only because both the contrast media and dye were injected under extreme pressures, opening tubes that under other circumstances would not be patent. HERS proved in these five patients (19% of the series) that there was no migration of ^{99m}Tc-HAM through the fallopian tubes, this being the probable cause for the infertility of these patients.

Even though HERS is a simple, safe, and accurate procedure, further studies will be necessary to establish its value as an additional and/or alternative study to other conventional procedures in evaluating tubal patency and its role as a functional radionuclide imaging modality in clinical gynecologic practice.

One indication for HERS would be to use it as a procedure to monitor the efficacy of sterilization procedures where the fallopian tubes are dissected or obstructed; or conversely to see if they are patent and open to transit after reconstructive surgery in patients that have been previously sterilized. In both cases the patient becomes her own control before and after the surgical procedure.

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